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Comparison of HPV-based assays with Papanicolaou smears for cervical cancer screening in Morelos State, Mexico

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Abstract

Objective: To compare the performance of human papillomavirus (HPV) assays with conventional Pap cytology for cervical cancer (CC) screening in Mexico.
Methods: Pap smears, self-collected vaginal specimens (SS) for HPV testing, and clinician-collected cervical specimens (CS) for HPV testing were obtained from 7868 women, aged 15–85 years old, attending CC screening at the Mexican Institute of Social Security (IMSS) between May and October, 1999. SS and CS specimens were screened for oncogenic HPV DNA by Hybrid Capture 2. Women who received cytological interpretations of atypical squamous cells of undetermined significance (ASCUS), and/or a positive HPV test were referred for colposcopy and histologic studies. The relative estimates for sensitivity, specificity and predictive values of each test were calculated using histological diagnoses of cervical intraepithelial neoplasia (CIN) grades 2 or 3, or CC histological diagnosis.
Results: Oncogenic HPV detection rate was 11.6% for SS, and 9.3% for CS. Pap smear abnormalities were observed in 2.4% of the women. Of 1147 women who had at least one abnormal test result, 88.5% underwent colposcopy, and 101 biopsy-confirmed CIN2/3 or cancer cases were identified. The relative sensitivity estimates for the Pap test, SS and CS were 59.4% (95% CI: 49.2–68.9), 71.3% (95% CI: 61.3–79.6), and 93.1% (95% CI: 85.8–96.9), respectively, while the specificities were 98.3% (95% CI: 98.0–98.6), 89.2% (95% CI: 88.5–89.9), and 91.8% (95% CI: 91.2–92.4), respectively. The positive predictive values of Pap, SS and CS were 36.1, 9.1 and 14.9, the colposcopy referrals needed to detect a case of CIN2/3 or cancer were 2.8, 11.0 and 6.7, respectively.
Discussion: Both HPV assays detected more cases of CIN2/3 or CC than Pap cytology alone. However, the HPV assays increased the number of colposcopy referrals. Our study suggests that HPV testing could be an effective way to improve the performance of CC screening.

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Introduction

During the last 40 years, Pap cytology has been the major means for cervical cancer (CC) screening. The

substantial reduction in mortality from this cancer that has been observed in most industrialized countries, has been attributed to Pap cytology-based early detection programs [1, 2]. However, CC continues to be a major health problem in developing countries, and is responsible for 235,000 deaths, worldwide every year [3]. Some of the highest incidence rates of CC in the world are found in Latin America and the Caribbean [4], where over 25,000 women die annually from this cancer [5]. Mortality rates in these countries have remained stable for the last 30 years [6], and this has been attributed mainly to the failure of cytology-based screening programs [7, 8].

Despite its successful track record in some countries, Pap cytology-based screening is far from perfect [9]. First, it is difficult to reach an adequate level of coverage and achieve a good follow-up of women with abnormal smears for diagnosis and treatment. Second, the test is dependent on the optimal collection of samples, and on the availability of well-trained cytopathologists. Third, the reading of cytology is highly subjective. Even in a well-organized CC screening program, such as the UK's, 50% of invasive cancers are detected in women who have been adequately screened [10]. In the US, false-negative Pap cytology results are one of the most frequent reasons for medical malpractice suits. Epidemiological studies have shown that in several developed countries with well-screened populations, CC mortality rates have reached a plateau and further reductions in mortality are not expected [11].

Given the high cost of frequent screening and the failures of Pap-based programs in many countries, especially in those with low healthcare expenditure, new alternatives for CC screening have been proposed. A possible solution may be to incorporate new technologies that have a higher sensitivity and acceptable specificity, and which may allow screening at longer intervals than with Pap cytology [12–14]. Based on the rationale that pre-invasive cervical lesions and invasive CC are caused by a persistent human papillomavirus (HPV) infection with one or more of the carcinogenic types, one of the proposals has been to incorporate HPV testing as an additional screening tool [15]. Some studies have suggested that HPV testing may be a cost-effective alternative for CC screening [16–19], particularly in settings where cytology is not readily performed [20].

The aim of this study was to evaluate whether HPV testing could be a more effective screening procedure than Pap cytology to detect pre-invasive lesions and CC within a regular CC screening program in a country with a high CC incidence and mortality. This paper reports a comparison of three CC screening methods: carcinogenic HPV assay in self-collected vaginal specimens (SS), in clinician-collected cervical specimens (CS), and Pap cytology.

Methods

Study population

This study was carried out within the regular population-based framework of the Instituto Mexicano del Seguro Social (IMSS) Cervical Cancer Screening Program (CCSP) in Morelos. IMSS is a governmental social security organization, providing health services to approximately 50% of the Mexican population. An estimated 95,000 women between the ages of 25 and 65 make up the target population of the IMSS CCSP in Morelos. All women attending CC screening services at any one of the 23 health units that make up the Morelos CCSP, were invited to join the study between May and October 1999. Women with a history of cervical intraepithelial neoplasia (CIN) 2/3 or CC, with a previous hysterectomy, or who were pregnant at the time were excluded. All participants provided informed consent. Detailed methodological aspects of this investigation have been reported elsewhere [21]. Table 1 includes some of the demographic and reproductive characteristics of the women who participated in the Morelos HPV Study. The mean age of the participants was found to be 42.5, and the mean age at first sexual intercourse was 19.2. Approximately 66% of the participants live in an urban area, while 34% reside in a semi-urban area. The mean number of live births among the study participants was 3.3.

Collection of specimens

Participants were asked to provide a SS for HPV testing. The nurses explained the self-collection procedure to the participants, and instructed them to insert a 15 cm cotton-tipped sterile dacron swab into their vagina until their fingers reached their labia, and then rotate the swab once to the left and once to the right. After removing the swab from their vagina, the women placed the sample in a specimen transport medium (STM) test-tube (Digene, Gaithersburg, MD).

At the same recruitment visit, trained nurses performed a pelvic examination to obtain a cervical specimen for a Pap smear, following standard procedures. After the Pap smear sample was collected, a second cervical specimen was collected for HPV DNA testing, using a conical cytobrush (Digene, Gaithersburg, MD) which was preserved in a STM test-tube. The SS and the CS specimens were kept at room temperature

Table 1. Demographic data of the Morelos HPV Study participants

Variable	n	Proportion
Age		
Mean = 42.5 years (SD = 13.4)		
<25	494	6.4
25–34	2034	26.3
35–44	2046	26.5
45–54	1601	20.7
55+	1557	20.1
Area of residence		
Urban	5072	65.6
Semi-urban	2660	34.4
Age at first sexual intercourse		
Mean = 19.2 years (SD = 3.8)		
<16	1061	13.7
16–19	3623	46.9
20+	3048	39.4
Number of pregnancies		
Mean = 4.2 (SD = 3.0)		
0–1	1050	13.6
2–3	2857	36.9
4–5	1838	23.8
6+	1987	25.7
Number of live births		
Mean = 3.3 (SD = 2.8)		
0–1	2224	28.8
2–3	2668	34.5
4–5	1449	18.7
6+	1391	18.0
Number of abortions		
Mean = 0.6 (SD = 1.0)		
0	4795	62.0
1–2	2529	32.7
3+	408	5.3
Number of cesarean births		
Mean = 0.4 (SD = 0.7)		
0	5778	74.7
1	1187	15.4
2+	767	9.9

SD – standard deviation.

and delivered once a week to the HPV laboratory at the National Institute of Public Health (INSP), where they were stored at −20 °C until analyzed.

Cytology interpretation

Cytological evaluations were performed using the local standard at the IMSS Cytology Center in Cuernavaca, Morelos. Smears were stained using a standard Papanicolaou method [22], and were classified according to the Bethesda System [23]: inadequate, normal, atypical squamous cells of undetermined significance (ASCUS), atypical glandular cells of undetermined significance (AGUS), low-grade squamous intraepithelial lesions (LSIL), high-grade squamous intraepithelial lesions (HSIL), adenocarcinoma *in situ* (AIS), squamous carcinoma, and adenocarcinoma. All the ASCUS, AGUS or definitely abnormal slides detected by the cytotechnicians were referred to a pathologist (EC) for final cytological diagnosis.

HPV testing

SS and CS specimens were tested for the presence of HPV DNA carcinogenic types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68, using the Digene Hybrid Capture 2 (HC2) microtiter assay, according to the manufacturer's instructions [24]. A special HPV laboratory was set up at INSP; training and standardization procedures were validated by Digene technicians. The laboratory personnel were blinded to the cytology results. The SS and CS HPV specimens were classified as positive if the relative light units/positive control (RLU/PC) ratio was ≥1 using the 1 pg/ml positive controls supplied in the kit.

Colposcopy and histology evaluations

Women who received cytological interpretations of ASCUS or worse and/or a positive SS or CS HPV test were referred for colposcopy and histologic studies, if indicated. There were 132 women who were referred but did not attend colposcopy services (11.5%), and were not considered in the analysis. All colposcopy procedures were performed by three colposcopists (MU, HM and AA) who were specially trained for this study by a senior colposcopist (DF), and lesions were graded using the Reid Index [25]. The colposcopists were aware of cytology and HPV results. They were instructed to biopsy all clinically suspicious lesions irrespective of the lesion severity. All women with an incomplete colposcopy (incomplete visualization of the endocervical canal) underwent an endocervical curettage for histological evaluation.

For quality control of the histological diagnosis, the local pathologist in Morelos (EC) and two pathologists at the Johns Hopkins School of Medicine (BR, MS) worked together to standardize the interpretation criteria for histological diagnoses. Sections from all biopsies were reviewed and diagnosed independently by two pathologists (EC, BR), without knowledge of the cytologic diagnoses, colposcopy data or HPV testing results. An additional reading was performed by a third pathologist (MS) in case of a disagreement and the final diagnosis was rendered by majority. All identified cases

of CIN2/3 or CC were treated following the standard treatment and follow-up procedures of the IMSS.

Analysis

The performance of the Pap smear, SS and CS HPV testing procedures for detecting histologically confirmed cases of CIN2/3 or CC was assessed by computing the point estimates and 95% confidence intervals (CI), of the relative sensitivity, specificity, positive and negative predictive values (NPV) for each test, as well as different combinations of the tests. These calculations were also performed using different RLU thresholds for the SS and/or CS collection methods (≥1, 2, 5, 20 and 100 pg/ml). A similar analysis using a different case definition was also carried out to evaluate the performance of these screening procedures for detecting CIN1 or worse.

Results

A total of 7868 women between the ages of 15 and 85 (median age 41 years) agreed to participate in the study. Overall, the response rate to the invitation to participate in the study was greater than 95%. The median age at first sexual intercourse was 18.7 years, with a median of 4.5 pregnancies. Thirty two percent of participants were postmenopausal, and 20% of participants were first time patients.

Of the 7868 recruited women, 136 (1.7%) had an inadequate Pap smear. Of these women, 11 had a positive HPV assay (1 CS; 3 SS; 7 CS and SS). These 136 women with inadequate Pap smears were excluded from the analysis, resulting in a total of 7732 participants with complete Pap and HPV test results.

Prevalence of cytology abnormalities

Among the 7732 Pap smears that were evaluated, 187 abnormal (ASCUS or worse) results (2.4%) were observed. All 12 women who received a cytological diagnosis of cancer were 45 years or older; whereas, only 43% (75/175) of the diagnoses of ASCUS/LSIL/HSIL were made in this age group.

HPV detection

The detection rates of high-risk HPV types, as detected in the SS and CS specimens, was 11.6% (95% CI: 9.6–13.9) and 9.4% (95% CI: 9.1–9.5), respectively. For both types of specimens, the detection rate was highest at the younger ages, decreased in age groups 35–54, and then increased again in women 55 years and older (Figure 1).

Colposcopic and histologic evaluations

Of the 1147 women with at least one abnormal test, we evaluated a total of 1015 women, by colposcopy resulting in a compliance rate of 88.5%. The colposcopy impressions of the 1015 compliant women were: normal (n = 583); CIN1 (n = 219); CIN2 (n = 68); CIN3 (n = 66); and cancer (n = 16); 63 women had an inadequate colposcopy.

A total of 432 women underwent histological evaluation, however, 2.6% of biopsies (n = 11) were inadequate for diagnosis. Nine of these eleven women were originally referred to colposcopy due to a positive cytology and/or HPV result (Pap = 2; SS = 4; CS = 1; SS + CS = 2; and Pap + SS + CS = 2), and their colposcopy impressions were CIN2 = 8 and CIN3 = 3; these cases were excluded from the analysis. The final histological diagnoses of the successfully evaluated women were: 272 normal; 48 CIN1; 89 CIN2/3 (CIN2 = 8 and CIN3 = 81); five AIS and adenocarcinomas; and seven squamous cell carcinomas. The distribution of cervical lesions according to specific combinations of screening tests is shown in Table 2.

Diagnostic performance of the tests

The relative sensitivity of the Pap smear to detect CIN 2/3 or CC was lower (59.4%; 95% CI: 49.2–68.9), as compared to 71.3% (95% CI: 61.3–79.6, $p = 0.008$) for SS, and 93.1% (95% CI: 85.8–96.9, $p = 0.0001$) for CS (Table 3). The relative specificities for both HPV tests were lower than for the Pap: 98.3, 89.2 and 91.8% for Pap, SS and CS procedures, respectively. The NPV were 99.5, 99.6 and 99.9% for Pap, SS and CS, respectively.

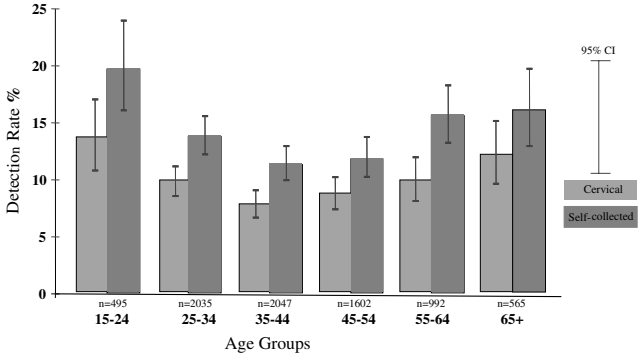

Fig. 1. High-risk HPV detection rates on clinical and self-collected samples, by age groups.

Table 2. Distribution of events according to specific combinations of screening tests

Screening tests			Histo-diagnosis								Total cases	Total biopsies
HPV SS	HPV CS	PAP	n	Colpo	Colpo/Nl	Histo/Ins	Normal	CIN I	CIN II	CIN III	Cancer	
+	+	+	104	98	12	2	31	11	2	33	7	42	84
+	+	–	416	370	227	2	93	21	3	22	2	27	141
+	–	+	4	3	2	0	0	0	1	0	0	1	1
+	–	–	371	331	240	4	79	6	0	2	0	2	87
–	+	+	27	26	3	0	8	2	1	10	2	13	23
–	+	–	173	144	86	1	41	4	1	10	1	12	57
–	–	+	52	43	13	2	20	4	0	4	0	4	28
–	–	–	6585	0	0	0	0	0	0	0	0	0	0
Totals			7732	1015	583	11	272	48	8	81	12	101	421

Colpo – compliant to colposcopy; Colpo/Nl – normal colposcopy impression; Histo/Ins – insatisfactory biopsy for histological diagnosis; Total/cases – total CIN II/III or cancer cases.

Table 3. Performance of the Pap smear, SS-HPV and CS-HPV testing strategies for cervical cancer screening

Test	Pap	HPV-SS	HPV-CS
Relative sensitivity	59.4	71.3	93.1
(95% CI)	(49.2–68.9)	(61.3–79.6)	(85.8–96.9)
Relative specificity	98.3	89.2	91.8
(95% CI)	(98.0–98.6)	(88.5–89.9)	(91.2–92.4)
Positive predictive value	36.1	9.1	14.9
(95% CI)	(28.9–44.0)	(7.2–11.4)	(12.2–17.9)
Negative predictive value	99.5	99.6	99.9
(95% CI)	(99.2–99.6)	(99.4–99.7)	(99.78–100.0)

The positive predictive values (PPV) of Pap, SS and CS were 36.1, 9.1 and 14.9, respectively (Table 3). The colposcopy referral ratio for detecting a CIN2/3 or CC case was 2.8 for Pap smear, 11.0 for SS and 6.7 for CS.

There were no substantial differences in the sensitivity or specificity values of the HPV tests or the Pap smear by age. The relative sensitivities for women 35 years and older were 66.0, 69.0 and 90.1% for Pap smear, SS and CS, respectively, and the relative specificities were 98.4% for Pap smear, 90.7% for SS and 93.2% for CS. The colposcopy referral rates were 2.7, 10.6 and 6.4 for Pap, SS and CS, respectively.

The performance of the CS-HPV test when using higher thresholds of HPV positivity (cutoff points of 2, 5, 20 and 100 pg/ml) resulted in a decreased sensitivity, and a relatively minor increase in specificity.

The relative sensitivity observed for the combination of SS and CS was 96.0% (95% CI: 89.6–98.7), and for the combination of Pap and CS it was 98.0% (95% CI: 92.3–99.7). The NPVs were 99.9 for the combination of SS and CS, and 99.9% for Pap with CS. None of sequential testing combinations offered acceptable relative sensitivities [SS + CS = 63.8%; Pap + SS = 20.7%; Pap + CS = 52.4%].

Discussion

Our findings indicate that HPV testing has a higher sensitivity to detect CIN2/3 or CC cases than the Pap smear and that the clinician-collected HPV test offers the best sensitivity for the detection of cervical lesions, with a higher NPV than Pap (99.9 *versus* 99.5, respectively). Overall, our findings suggest that CS-HPV testing may be an effective strategy to improve primary CC screening in Mexico.

The IMSS CCSP in Morelos is considered one of the best by local standards in Mexico. For this reason, the observed differences in sensitivity between HPV testing and the Pap smear may be even larger in other CCSPs throughout the country.

One limitation of the study is that only women with abnormal assays were referred to colposcopy. This prevented us from accurately estimating the false-negative rates of the tests and correcting for underdetection of actual cases in the study population. A study by Schiffman *et al.* in Costa Rica [17] did not identify any cases of disease among 138 randomly selected women having a normal Pap and a negative HPV testing that were referred to colposcopy. Also, given the high

sensitivity of combined HPV testing and Pap smear observed in other studies where all women were biopsied (Pap plus HPV by HC2 had a sensitivity of 100%; Belinson *et al*., unpublished data). In addition, it is likely that the incorporation of a third test may have compensated for this bias. Thus we believe that verification bias in our data will be small, if any, and unlikely to explain our results.

The colposcopy compliance in the study was relatively high (88.5%), and there were minor differences in compliance rates for the specific test results (83.2, 89.2 and 82.7%, for CS, SS and Pap, respectively). However, when cytology abnormalities were present, independent of HPV test results (SS, CS or both), the biopsy rates were 65% compared to 40% among women who had only HPV positive results (Table 2). These differences may be attributed to a lower detection rate of abnormalities by colposcopy in HPV-positive women. The physician's decision to collect a biopsy may also have been influenced by the test results leading to a verification bias. However, if our estimates were actually affected by this potential bias, we would expect the differences in sensitivity between the cytology and HPV methods to be even larger than those that were actually observed. These estimates were corrected for potential verification bias secondary to follow-up loss or unconfirmed cases (due to inadequate samples), based on the observed rates of confirmed cases for each single or combined screening procedure, as suggested in previous studies [16]. With the corrected estimates we observed a slight reduction in the sensitivities of the tests: 57.9% (95% CI: 48.3–66.9), 70.4% (95% CI: 61.0–78.3), and 90.7% (95% CI: 83.4–95.0) for Pap, SS, and CS, respectively. The corrected specificities were 98.6 (95% CI: 98.1–99.2), 90.5 (95% CI: 94.5–96.1), and 93.2 (95% CI: 92.1–93.3) for Pap, SS, and CS, respectively.

The CS-HPV test performed substantially better than the Pap smear. CS-HPV testing was 57% more effective at detecting CIN2/3 or CC than cytology, and 119% more effective at detecting CIN1 or worse. The CS-HPV test performance observed in this study is comparable to most previous reports [17–20], except for Ratnam *et al*. in Newfoundland who reported a sensitivity of 85% [16]. An explanation for this discrepancy is that these researchers used, the first generation of HC for HPV testing, which has a lower sensitivity than HC 2 [18].

The lower PPV of CS-HPV testing compared to that of the Pap smear (14.9% *versus* 36.1%) resulted in a high colposcopy referral rate. A CS-HPV-based screening strategy could result in a more than doubling of the number of women referred for colposcopy. However, the HPV-positive disease-negative women may represent a group at higher risk of future disease, and therefore require a closer monitoring. Additionally, the high NPV attained by CS-HPV offers the potential of extending the screening intervals. Lengthening the screening intervals could help offset the costs of additional colposcopy services generated within CS-HPV screening strategy [26, 27].

No substantial differences were observed in the performance of the CS-HPV testing procedure by age categories. These findings are consistent with those by Wright *et al*., in South Africa [20] and Schiffman *et al*. in Costa Rica [17]. Also, the use of different cutoff points as a basis for colposcopy referral did not offer appreciable advantages. The relatively low improvement in specificity derived from a higher threshold of HPV positivity may not compensate for the considerable penalty imposed on the sensitivity rates in both assays. The cutoff point of one pg/ml, that has been used in some other studies [17–20], seems to be an optimal cutoff for our population as well.

The cytology sensitivity that we observed is somewhat lower than what has been previously reported [17, 18, 20]. This may be due to the fact that better Pap quality control procedures were implemented in these studies. For our study we used the standard procedures of the Cytology Center of the IMSS CCSP in Morelos. The age distribution of cytological abnormalities in the study population was similar to the age distribution of women with abnormal cytology during the past three years in the Morelos CCSP [21]. The low Pap smear sensitivity in our study may explain, in part, the low effectiveness of the current cytology-based screening program in Mexico [28].

In our study the SS-HPV test detected more cases of CIN2/3 or CC than the Pap smear. Our sensitivity estimates (71.3%, 95% CI: 61.3–79.6) are comparable to those reported by Wright *et al*. [20] in South Africa (66.1%, 95% CI: 52.1–77.8). The observed lower performance of SS-HPV compared to CS-HPV is also consistent with previous studies in clinical settings [29], and with the results of the screening program in South Africa [20]. The potential for improving the 'quality' of specimens obtained using self-collection procedures needs to be further investigated in order to improve its performance. However, SS-HPV testing may offer some additional advantages that should also be considered. In many settings women are reluctant to undergo a pelvic examination, so the incorporation of this procedure could offer an important alternative for women who might be reluctant to accept cytology or CS-HPV screening [7, 30]. However, although self-sampling may provide a more acceptable method of screening and could help to improve coverage, it could also increase the number of women who might chose self-sampling

over CS-HPV testing despite the fact that it is less sensitive. In order to improve coverage and not compromise the efficacy of screening, this potential preference, as seen in our study [31], should be taken into account when designing a program that incorporates HPV testing.

Our findings confirm the potential benefits of incorporating HPV testing into CC primary screening procedures. The CS-HPV test is an effective alternative that can be used to increase the sensitivity of CC screening in Mexico. In summary, our study suggests that HPV testing could be effective in reducing the CC burden in Mexico. However, future longitudinal studies are needed to evaluate its performance in the context of population based screening programs. Inclusion of HPV testing in prevention efforts is not a substitute for public health promotion efforts urgently needed to improve CC screening programs in the region.

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